## Original Article:

# Effect of block-replacement regimen on bone mineral density and biochemical markers in patients with thyrotoxic bone disease

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## ABSTRACT

**Introduction:** Abnormal bone metabolism in patients with thyrotoxicosis is well documented, but time-course of recovery remains poorly understood.

**Objective:** To evaluate changes in bone density and bone metabolic parameters in thyrotoxicosis before and after therapy.

**Methods:** Forty patients with thyrotoxicosis (11 males; mean age 35.5 ± 9.9 years) rendered euthyroid with methimazole and maintained on block-replacement therapy were followed up for six months at a tertiary care setting. Of these, 21 had completed follow-up evaluation. Bone mineral density (BMD) at lumbar spine (LS), hip and biochemical markers were estimated before and after therapy.

**Results:** At LS, 21% women and 35% men had a Z score less than –2. At the neck of femur (NOF),17% women and 18% men had Z score less than -2. LS was more severely affected ($p < 0.001$). BMD was similar among patients who were 25-hydroxy vitamin D [25(OH)D] deficient,[25(OH)D < 20 ng/mL (<50 nmol/L)], insufficient [25(OH)D 20 - 30 ng/mL (50-75 nmol/L)] or sufficient [25(OH)D > 30 ng/mL (>50 nmol/L)]. Serum $T_4$ correlated negatively with Z-scores at LS, NOF, trochanter and Ward's area. There was improvement in BMD ($p<0.01$) and Z scores ($p<0.01$) at all levels post-treatment with higher increase at NOF ($p=0.046$). A significant decrease in serum calcium, 24 hour urinary calcium and phosphate and the fractional excretions of calcium and phosphate was also seen, while serum N-tact PTH levels increased.

**Conclusions:** Correction of hyperthyroidism plays a pivotal role in improving the BMD and biochemical parameters.

***Key words:*** *Bone formation and resorbtion , Thyroid, Vitamin D, block-replacement, Methimizole*

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## INTRODUCTION

The adverse effects of hyperthyroidism on the skeleton were known even before the advent of satisfactory treatment for hyperthyroidism. Fracture risk is increased in hyperthyroidism.[1,2] The extent of the reduction in bone mineral density (BMD) ranges from 10% to 20%.[3-7] However reversibility of bone loss with therapy is unclear.[3-7] Despite the variable BMD, a history of overt hyperthyroidism is a risk factor for hip fracture later in life.[8] Increased risk of spine and forearm fractures has been reported in patients treated with radioiodine but not in those treated with methimazole.[9]

Increased bone resorption leads to negative calcium balance.[10] Hypercalcaemia occurs in up to 8% of patients.[10] Hypercalcaemia suppresses the secretion of parathyroid hormone (PTH), leading to hypercalciuria, negative calcium balance and reduced conversion of 25-hydroxyvitamin D [calcidiol, 25(OH)D] to calcitriol.[11] The decline in calcitriol production is compounded by an increase in calcitriol metabolism induced by hyperthyroidism,[12] leading to diminished intestinal calcium (and phosphorous) absorption and faecal calcium loss. There is paucity of data from Indian subcontinent on this topic. A previous study reported osteopenia in 32% , and osteoporosis in 60% with improvement in bone mass 1 year after control of thyrotoxicosis.[13] Dhanwal et al[14] compared the effect of vitamin D deficiency on BMD in thyrotoxicosis patients. They found that in the vitamin D-deficient group, the mean BMD T-scores were in the osteoporotic range at hip and forearm (–2.65 ± 1.13 and –3.04 ± 1.3) and in

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the osteopenia range at lumbar spine (–1.83 ± 1.71) and BMD (g/cm$^2$) at the hip and forearm was lower in the vitamin D-deficient group compared with those in the vitamin D-sufficient group.

In the background of widely prevalent vitamin D deficiency in Indian subcontinent[15-17] hyperthyroidism could have deleterious effects on bone mineral homeostasis. Peak bone mass in Indians is low, resulting in low bone mineral density in adult life.[18] There is paucity of data in hyperthyroid patients with regard to biochemical markers of bone mineral metabolism and BMD before and after therapy.

Hence, the objectives of the study were: (i) to study the bone mineral metabolic markers in patients with thyrotoxicosis before and after anti-thyroid drug therapy; (ii) to study the BMD in patients with thyrotoxicosis before and after anti-thyroid drug therapy; and (iii) to study the impact of various factors like severity of thyrotoxicosis, duration of symptoms of toxicosis, parity, duration of lactation, vitamin D status etc., on BMD in patients with thyrotoxicosis.

## MATERIAL AND METHODS

Forty patients, diagnosed to have clinically and hormonally proven thyrotoxicosis, were included in this prospective study conducted in the Department of Endocrinology and Metabolism, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, India, from March 2007 to March 2008. Informed consent was obtained from all participants and the study was approved by institute ethics committee.

All treatment naïve patients presenting with a 3-6 month history of weight loss, palpitations and clinical manifestations of thyrotoxicosis were included in the study. Thyrotoxicosis was confirmed hormonally by an elevated total serum thyroxine [$T_4$ > 135 ng/ml (174 nmol/L)], an undetectable serum thyroid stimulating hormone (TSH) less than 0.15 mU/L and a $^{99m}$Tc thyroid scan showing an increased uptake (>4%). Patients with thyrotoxicosis who are already on therapy, patients being treated with oestrogens, thiazide diuretics, calcium, or vitamin D for at least 12 months before enrolment in the study, patients with rheumatoid arthritis, diabetes mellitus, liver disease; patients with family history of osteoporosis, smokers and chronic alcoholics were excluded from the study.

### Study procedure

In all patients, a detailed history was taken and clinical examination was performed. Specific attention was given to: total duration of symptoms, severity of the disease as per Wayne's thyroid score (<11= nontoxic, 11 to 19 = equivocal, >19= toxic),[19] parity (the total number of pregnancies reaching 28 weeks of gestation or more), duration of lactation (months) and sunlight exposure (total minutes of sunlight exposure per day averaged from a record over a week) and the body surface area exposed (using "rule of nines" in burns).[20]

### Laboratory investigations

The thyroid hormones were measured using the following radioimmunoassay (RIA) kits and immunoradiometric (IRMA) kits from Bhabha Atomic Research Center (BARC) Mumbai: $T_4$ by RIAK-5A (RIA), total serum triiodothyronine ($T_3$) by RIAK-4A (RIA) and serum TSH by IRMAK-9 (IRMA) kits. The minimum and maximum detectable limits for serum $T_4$, $T_3$, and TSH were 15-240 ng/mL (19-309nmol/L), 0.3-2.4 ng/mL (461- 3687 pmol/L) and 0.15-50 mU/L respectively. A $^{99m}$Tc radionuclide thyroid scintigraphy was performed using 3 to 5 millicurie of technetium using a standard protocol after 20 minutes.[21] Anterior, right anterior oblique and left anterior oblique views were taken to measure the radioactive uptake, expressed as percentage uptake. Levels of anti-thyroid peroxidase (anti-TPO) antibodies were estimated at the time of diagnosis by IRMA using kits from Diasorin (AB-TPOK-3 - P002046), the limits of detectability being 15 - 1000 AU/mL.

Serum calcium, phosphorus, alkaline phosphatase (SAP), creatinine and albumin were determined on CX9 autoanalyzer (Beckmann Coulter Synchron Brea, CA) using commercial kits. The 25(OH)D was measured by RIA (DiaSorin, Stillwater, MN; Catalog no. 68100E), minimum detectable limit being 5 ng/mL (12 nmol/L). N-

tact PTH was measured by IRMA (DiaSorin; Catalog no. 26100) with minimal detectable limit being 13.8 ng/L. The subjects were classified as vitamin D deficient, insufficient, or sufficient on the basis of 25(OH)D concentrations of less than 20 ng/mL (50 nmol/L), 20-30 ng/mL(50-75nmol/L), and greater than 30 ng/mL (>75 nmol/L), respectively.[22-24]

The 24-hour urine samples for calcium, phosphorous, creatinine were collected in calcium free containers. Patients were categorized as hypercalciuric (> 4 mg/kg body weight) or normocalciuric (< 4 mg/kg body weight) based on urinary calcium excretion.[25] The fractional excretion of phosphate and tubular transport maximum for phosphorus (TmP/glomemlar filtration rate (GFR) were determined and analysed using Bijovet index/nomogram [normal TmP/GFR= 2.8-4.4 mg/dL(0.9-1.42mmol/L)].[25]

**Bone mineral density**

Baseline BMD was measured by DXA scans (Discovery A, Hologic inc. version-12.6.1 USA) at the time of diagnosis before initiating the patients on anti-thyroid drugs. Lumbar spine (LS) anterior-posterior L1-L4 and hip (femoral neck, the Ward triangle, intertrochantric region and trochanter) were scanned for measurement of BMD. The values of BMD measurements are expressed in g/cm$^2$. For BMD reporting in females prior to menopause and in males younger than age 50 years, Z-scores were used. A Z-score of –2.0 or lower is defined as "below the expected range for age" and a Z-score above –2.0 as "within the expected range for age."[26] BMD reports were analyzed using manufacturer's Asian database.

**Intervention**

Antithyroid therapy was initiated with methimazole (20-40 mg) after baseline studies and was continued to maintain euthyroid status, defined as return of T4 to normal range (55-135 ng/mL). These patients were later started on a block-replacement regimen of prescribing a full dose of a thionamide drug and adding $T_4$ supplements (75-150 µg/day) to prevent the patient from becoming hypothyroid. Propranolol was used only during the first month for patients with clinically significant sympathoadrenergic symptoms. Liver toxicity was monitored for by estimating prothrombin time and serum albumin before and after treatment.

All patients underwent a repeat assessment of biochemical parameters along with BMD 6 months after they became euthyroid. The drift of the BMD machine had a coefficient of variation (CV) of 0.385% throughout the duration of study.

**Statistical analysis**

Statistical analysis was performed using statistical package for social sciences (SPSS) for Windows, release 10.0.1 (SPSS Inc, Chicago, Illinois, USA). Data were presented as mean ± standard deviation (SD). Continuous variables were analyzed with independent sample t-test and paired t-test as appropriate. Significance was assumed at a p-value less than 0.05. BMD among vitamin D deficient, insufficient and sufficient groups were compared using one way analysis of variance (ANOVA). Spearman's correlation co-efficient for linear correlation was calculated. Linear regression analysis was used to obtain threshold of thyroid hormones above which toxic effects were exerted.

## RESULTS

**Baseline data**

Forty patients with clinically and biochemically confirmed thyrotoxicosis were included in the study. Their mean age was 35.5 ± 9.9 years; there were 11 males. The genders were not different from each other with respect to the age of the patients enrolled (Table 1). All patients had diffuse thyroid enlargement. The mean Wayne's clinical score for thyrotoxicosis, among women and men was 24 ± 6.4 and 24 ± 7.8 respectively, at the time of diagnosis (p=NS). All patients had elevated serum $T_3$, $T_4$, undetectable serum TSH levels and diffusely increased uptake on $^{m99}$Tc scintigraphy (Table 1). Twenty one patients completed the six-month follow-up; 19 were lost to follow-up. Except for duration of sun exposure which was higher in women (p=0.03), none of the other variables were significantly different between the genders. Only one patient wore a veil (burkha), for whom surface area of sun exposure was considered as zero. Mean 24-hour urinary calcium

excretion was higher among women but other biochemical indices, including serum 25(OH)D, were not different between the genders (Table 1).

Hypercalcaemia [serum calcium >10.5mg/dL (2.62mmol/L)] was present in 10 (25%) of patients. Hypercalciuria was noted in 17 (42.5%) of patients. Seven out of 10 patients (70%) with Hypercalcaemia had hypercalciuria. Among hypercalciuric patients(n=17), 41% had hypercalcemia.

Vitamin D deficiency was seen in 23(58%) patients and vitamin D insufficiency was seen in 14 (34%)patients; normal serum 25(OH)D was evident in only 3 (8%)patients. Baseline BMD and Z- scores of LS and femur in women and men are shown in Table 2. Lumbar spine Z- scores were more severely affected compared to femur ($p < 0.001$) in patients with thyrotoxicosis at the time of diagnosis. Both genders were equally affected. Distribution of patients based on their Z-scores at various levels in lumbar spine is shown in Figure 1. Eighty six per cent of women and 64% of men had Z-score of less than –1 at LS at the time of

**Table 1: Gender-wise comparison of clinical and biochemical characteristics of 40 patients with thyrotoxicosis at recruitment**

| | Women (n=29) | Men (n=11) |
|---|---|---|
| Age (years) | 34.7 ± 9.5 | 37.5 ±11.0 |
| Duration of symptom (months) | 10.3 ± 9.4 | 9.7 ± 7.7 |
| Parity (no.) | 1.66 ± 0.82 | NA |
| Lactation (months) | 22.0 ± 21.0 | NA |
| Duration of sunlight exposure (min/day) | 72.7 ± 93.5 | 31.8 ± 19.9* |
| Area exposed to sun (as % of total body surface area) | 27.3 ± 1.7 | 25.4 ± 3.6 |
| Wayne's Score | 24 ± 6.4 | 24 ± 7.8 |
| Weight (kg) | 48.4 ± 8.1 | 61.0 ± 9.7† |
| BMI (kg/m$^2$) | 20.39 ± 3.73 | 21 ± 2.7 |
| $T_3$ (ng /mL) | >2.4 ± 0 | >2.4 ± 0 |
| $T_4$ (ng/mL) | 242.4 ± 58.1 | 231.8 ± 39.2 |
| TSH (mIU/mL) | <0.15 ± 0 | <0.15 ± 0 |
| $^{99m}$Tc uptake (%) | 11.2 ± 5.2 | 9.9 ± 2.6 |
| Anti-TPO (AU/mL) | 374.0 ± 389.9 | 326.5 ± 345.5 |
| Prothrombin time (seconds) | 15.2 ± 1.8 | 14.9 ± 1.4 |
| Dose of methimazole (mg/day) | 30 ± 2.7 | 31.8 ± 4.1 |
| Serum albumin (g/dL) | 3.7 ± 0.25 | 3.8 ± 0.5 |
| Corrected calcium (mg/dL) | 10.3 ± 0.6 | 10.4 ± 0.55 |
| Serum phosphorus (mg/dL) | 3.7 ± 0.7 | 3.9 ± 1.0 |
| Serum Alkaline Phosphatase (IU/L) | 113.2 ± 31.2 | 93 ± 37.1 |
| Serum 25(OH)D (ng/mL) | 18.9 ± 6.7 | 17.1 ±9.5 |
| Serum N-tact PTH (pg/mL) | 26.1 ± 11.3 | 28.5 ± 10.6 |
| 24-hour Urinary calcium (mg/day) | 242.9 ± 174.1 | 181.6 ± 137.2 |
| 24-hour Urinary calcium (mg/kg of bodyweight) | 5.3± 4.2 | 2.9 ± 2.04* |
| 24-hour Urinary creatinine (mg/day) | 555.7 ± 161.7 | 840.1 ± 467.3 |
| 24-hour Urine phosphorus (mg/day) | 512 ± 236.7 | 556.5 ± 245.7 |
| Fractional excretion of calcium (%) | 2.98 ± 3.2 | 2.65 ± 3.4 |
| Fractional excretion of phosphorus (%) | 15.9 ± 9.8 | 22.9 ± 23.7 |
| TmP/GFR (mg/dL) | 3.2 ± 0.7 | 3.0 ± 1.3 |

Data presented as mean ± SD.

*= $p < 0.05$, † = $p = 0.01$

***For normal values, see text.***

Anti-TPO= anti-thyroid peroxidease; PTH = parathyroid hormone; 25(OH)D = vitamin D; TmP= tubular transport maximum for phosphorus; GFR = glomerular filtration rate;NA-Not Applicable

diagnosis. At LS, 21% women and 35% men had Z-score less than –2. Distribution of patients based on their Z-scores in femur is shown in Figure 2. In the neck of femur (NOF), 60% of men and 66% of women had Z-score less than –1; 17% women and 18% men had Z-score less than –2. BMD was similar among various vitamin D groups.

Negative correlation was noted between BMD and severity of symptom score, parity and lactation. Serum $T_4$ correlated negatively with Z-scores of LS, NOF, trochanter and Ward's area. Serum calcium, serum phosphorous, 24-hour urinary calcium and phosphorous did not show any correlation with Z-scores or BMD of LS or hip. On linear regression analysis, calculated cut-offs of $T_4$ for one-standard deviation fall in age-and sex-matched BMD are 128 ng/mL (165 nmol/L) [Z-score= –0.243 + (–0.006 × T4), $r^2$=0.093] for LS, 180.7 ng/mL (233 nmol/L) [Z-score=0.082 + (–0.005 × T4), $r^2$=0.110] for NOF and 218.7 ng/mL (282 nmol/L) {Z score= –0.311 + (–0.003 × T4), $r^2$=0.037} for total hip.

**Table 2: Gender-wise comparison of BMD and Z-scores in 40 patients with thyrotoxicosis at the time of recruitment.**

| Region | Baseline bone mineral density | | | |
|---|---|---|---|---|
| | Women (n = 29) | | Men (n=11) | |
| | BMD (g/cm$^2$) | Z-score | BMD (g/cm$^2$) | Z-score |
| Lumbar spine (total) | 0.823 ± 0.098 | –1.8 ± 0.8 | 0.926 ± 0.167 | –1.3 ± 1.5 |
| Neck of femur | 0.691 ± 0.095 | –1.2 ± 0.8 | 0.75 ± 0.127 | –1.0 ± 0.9 |
| Greater trochanter | 0.571 ± 0.075 | –1.2 ± 0.7 | 0.66 ± 0.141 | –0.8 ± 1.1 |
| Intertrochantric region | 0.905 ± 0.118 | –1.2 ± 0.8 | 1.08 ± 0.16* | –0.6 ± 0.9 |
| Total hip | 0.772 ± 0.098 | –1.2 ± 0.9 | 0.88 ± 0.15** | –0.7 ± 0.9 |
| Ward's area | 0.567 ± 0.137 | –1 ± 1.0 | 0.63 ± 0.16 | –0.6 ± 1.1 |

Data presented as mean ± SD;
* = p< 0.01 † = p< 0.05;
BMD = bone mineral density;
Z-score = standard deviation score calculated from age and sex matched healthy controls
Comparison of areal bone mineral density (g/cm$^2$) of men Vs women at baseline.

**Figure 1:** Percentage of patients having Z-scores in specified ranges at each lumbar verterbra in women and men separately at the time of recruitment

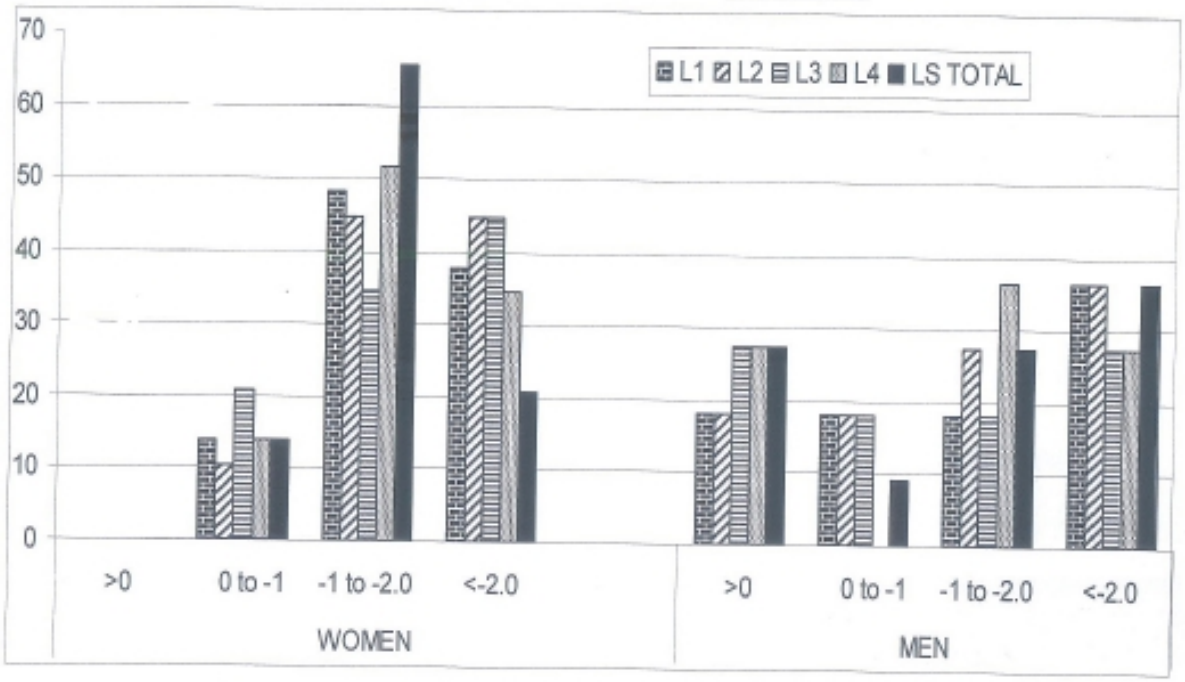
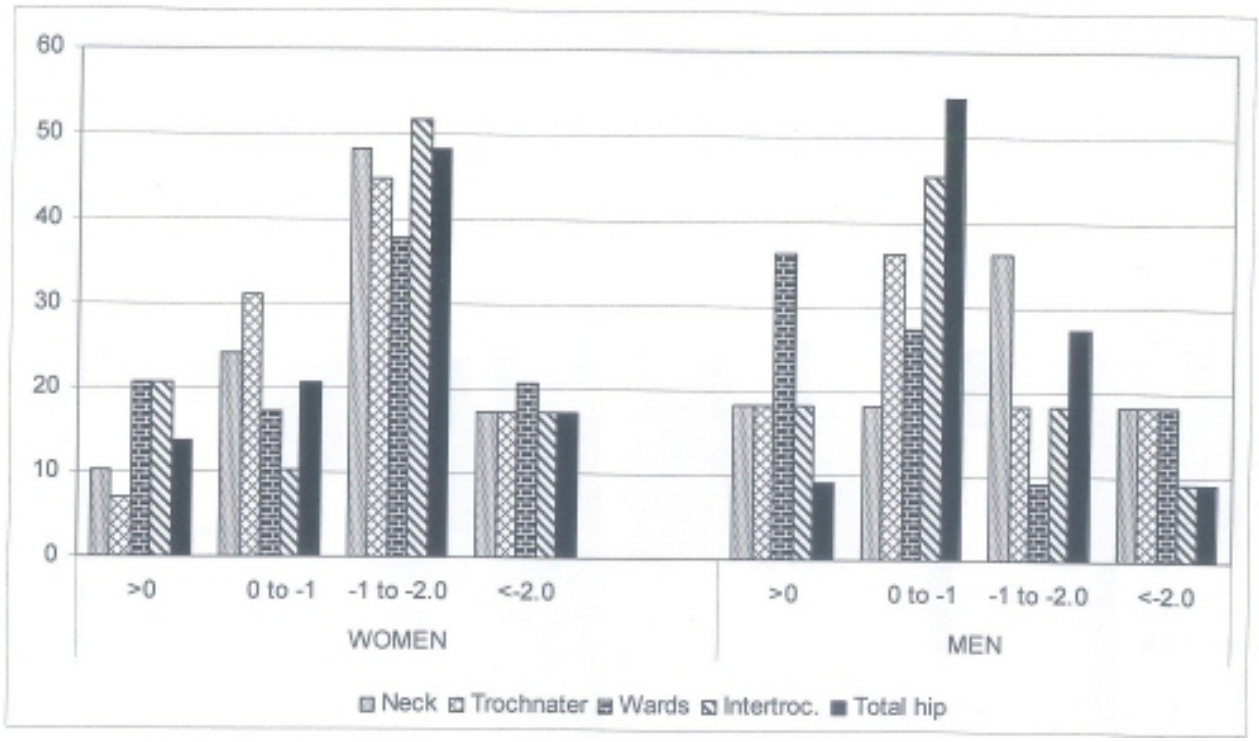


**Figure 2:** Percentage of patients having Z-scores in specified ranges at each region of hip in women and men separately




**Post-treatment**

On follow-up, 14 women and 7 men underwent a repeat BMD and assessment of biochemical markers 6 months after achievement of euthyroid state. The mean duration of treatment to achieve euthyroid state was 6.5 ± 1.2 weeks. There was no significant difference between males and females. Following treatment of thyrotoxicosis significant improvement was noted in body weight and body mass index (BMI).

**Biochemical markers**

Comparison of biochemical indices pre-and-post treatment is shown in Table-3. Post-treatment $T_4$ decreased ($p<0.001$) and TSH increased. Post-treatment, the whole group had a significant decrease in serum calcium ($p=0.001$), 24-hour urinary calcium and phosphorus excretion, and fractional excretion of calcium and phosphorous. There was no change in serum 25(OH)D levels with antithyroid therapy. The pos-treatment serum N-tact PTH showed a significant increase ($p<0.005$). There was a decrease in serum calcium, 24-hour urinary calcium excretion and fractional excretion of phosphorous in women (Table 3). On the other hand, there was a decrease in 24-hour urinary phosphorous excretion in men (Table 3) that was not seen in women. Post-treatment rise in PTH was observed only in women.

Among patients who had completed the 6-month follow-up (n=21), 5 had Hypercalcaemia and 9 had hypercalciuria at recruitment. Eighteen of these 21 patients had a fall in serum calcium levels while 3 had a mild rise. While one patient had a post-treatment elevated serum calcium [10.6 mg/dl (2.64 mmol/L), i.e., a 1.5% rise from baseline]; two others had serum calcium levels less than 10.5 mg/dL (2.62 mmol/L), albeit with a 1.6% and 3.4% rise from baseline. In these 18 patients, a fall in 24-hour urinary calcium excretion was observed. Of the 9 patients who had hypercalciuria at diagnosis, 7 became normocalciuric and 2 continued to be hypercalciuric despite having fall of 54% and 65% respectively. Three patients, who were normocalciuric initially, had a mild rise in urinary calcium excretion, but still remained normocalciuric. When patients were analyzed together, there was improvement in BMD and Z-scores at all sites of LS and femur. Similar improvement was seen post-treatment in women.

In men, there was improvement in BMD only at LS (total), L1 vertebra, NOF and trochanter (Table 4) while improvement in Z-scores was noted at LS (total), L1, L2, NOF, trochanter and intertrochantric region (Table-4). Percentage increase in BMD was more at femur (10% ± 8%) as compared to lumbar spine (7% ± 6%). There was no difference in improvement in BMD between the genders. At LS post-treatment, 21% of women and 14% of men still had Z-scores less than –2 while none had such low Z-scores at NOF.

## DISCUSSION

In the present study, Z-scores less than –2 (below the expected range for age) were seen in 21% and 35% at LS and 17% and 18% at femur in women and men respectively. A reduction in BMD of 7.4%-12% has been seen at LS and 13% at trochanter in various studies.[7,27] Two studies using single photon absorptiometry reported a reduction in bone density of 12% to 28% in hyperthyroid patients, which normalized after treatment.[3]

Usefulness of the Wayne's scoring system forsymptoms of thyrotoxicosis has been undermined because of the easy availability of the biochemical markers of thyroid function. However, we found useful negative correlation of Wayne's score with BMD at LS, greater trochanter and Ward's area. Wayne's score is thus a useful and simple clinical pointer to identify patients at risk for thyrotoxic bone disease. Every case of thyrotoxicosis with high Wayne's score should be evaluated for bone disease.

The duration of symptoms of thyrotoxicosis is not known to correlate with bone density at any site.[29] As already known, weight and BMI showed a positive correlation with bone density at LS, greater trochanter, intertrochanteric region and total hip ($p < 0.05$). The weight loss related to thyrotoxicosis may also contribute to the reduction of bone density. The positive effect of weight and BMI on BMD is known in general population, weight having a stronger association with bone mass, probably due to mechanical factors.[29]

In our study parity showed a significant negative correlation with BMD at both LS and hip, an association not supported in literature.[30] Some

**Table 3: Comparison of biochemical parameters pre-and at 6-months after achieving euthyroid state.**

| | Women (n=14) | | Men (n=7) | |
|---|---|---|---|---|
| | **Pre-treatment** | **Post-treatment** | **Pre-treatment** | **Post-treatment** |
| $T_4$ (ng/mL) | 246.4 ± 64.4 | 115.6±33.3‡ | 237.9 ± 41.7 | 112 ± 4.8‡ |
| TSH (mIU/mL) | <0.15 ± 0 | 1.4 ± 2.0* | <0.15 ± 0 | 2.5 ± 3.5 |
| Serum albumin (g/dL) | 3.6 ± 0.3 | 3.8 ± 0.2† | 3.7 ± 0.6 | 3.9 ± 0.3 |
| Corrected serum calcium (mg/dL) | 10.5 ± 0.44 | 9.85 ± 0.53† | 10.4 ± 0.54 | 9.9 ± 0.146 |
| Serum phosphorus (mg/dL) | 3.75 ± 0.70 | 3.65 ± 0.58 | 3.84 ± 0.99 | 3.77 ± 0.55 |
| Serum alkaline phosphatase (IU/L) | 103 ± 23 | 116 ± 29 | 107.2 ± 40.2 | 143.20 ± 66.3 |
| Serum 25(OH)D (ng/mL) | 20.5 ± 6.7 | 17.2 ± 6.4 | 17.1 ± 11.5 | 17.3 ± 11.6 |
| Serum N-tact PTH (pg/mL) | 23.5 ± 9.2 | 44.4 ± 13.6* | 29.4 ± 12.1 | 63.5 ± 30.9 |
| 24-hour Urinary calcium (mg/day) | 228 ± 157 | 128.5 ± 82.6* | 154.7 ± 105 | 108.7 ± 47.9 |
| 24-hour Urinary calcium (mg/kg of bodyweight) | 5.4 ± 4.7 | 2.57 ± 1.78† | 2.46 ± 1.61 | 1.51 ± 0.59 |
| 24-hour Urinary phosphorus (mg/day) | 509 ± 204 | 331.2± 192.4 | 555.3 ± 260.1 | 370.3± 181.6* |
| Fractional excretion of calcium (%) | 2.7 ± 2.6 | 1.4 ± 1.1* | 1.7 ± 1.0 | 1.1 ± 0.5* |
| Fractional excretion of phosphorus (%) | 15.9 ± 7.8 | 10.2 ± 7.4* | 18.9 ± 12.7 | 9.5 ± 3.8 |
| TmP/GFR (mg/dL) | 3.2 ± 0.7 | 3.3 ± 0.6 | 3.1 ± 1.0 | 3.4 ± 0.5 |

Data presented as mean ± SD

Comparison of biochemical indices pre- and post-treatment in the same sex group:

* = $p < 0.05$, † = $p < 0.01$, ‡ = $p < 0.001$

PTH = parathyroid hormone; 25(OH)D = vitamin D; $T_4$ = tetraidothyronine; N-tact PTH = intact PTH;

TmP = tubular transport maximum for phosphorus; GFR = glomerular filtration rate

**Table 4:Comparison of body weight, BMD and Z-scores at baseline and at 6-months after achieving euthyroid state**

| Parameter | Women (n=14) | | Men (n=7) | |
|---|---|---|---|---|
| | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| Weight (Kg) | 47.8± 9.5 | 53.0± 9.8‡ | 61.3± 11.5 | 71.6± 9.9† |
| BMI (Kg/m$^2$) | 20.17± 4.63 | 22.39 ± 4.83‡ | 20.81± 2.92 | 24.41± 2.73† |
| BMD spine (g/cm$^2$) | 0.806± 0.094 | 0.865 ± 0.109† | 0.938 ± 0.152 | 0.990± 0.122* |
| BMD NOF (g/cm$^2$) | 0.686 ±0.085 | 0.748± 0.094‡ | 0.731± 0.099 | 0.775 ± 0.085* |
| BMD trochanter(g/cm$^2$) | 0.566 ±0.075 | 0.627 ±0.070‡ | 0.638± 0.118 | 0.689± 0.092* |
| BMD Ward's triangle(g/cm$^2$) | 0.552± 0.128 | 0.609 ±0.142‡ | 0.592± 0.135 | 0.633± 0.112 |
| BMD intertrochanteric region (g/cm$^2$) | 0.903± 0.138 | 0.993± 0.118‡ | 1.070 ± 0.141 | 1.125± 0.131 |
| BMD femur (g/cm$^2$) | 0.769± 0.108 | 0.848 ±0.091‡ | 0.888± 0.117 | 0.910± 0.173 |
| **Z-SCORES** | | | | |
| total spine | –1.9 ± 0.7 | –1.4 ± 0.9† | –1.3 ± 1.4 | –0.8 ± 1.2* |
| NOF | –1.2 ± 0.7 | –0.6 ± 0.8‡ | –1.1 ± 0.8 | –0.7 ± 0.7* |
| Trochanter | –1.2 ± 0.7 | –0.6 ± 0.7‡ | –0.9 ± 0.9 | –0.5 ± 0.7* |
| Ward's area | –1.1 ± 0.9 | –0.5 ± 1.0‡ | –0.7 ± 0.9 | –0.4 ± 0.8 |
| Intertrochanteric region | –1.2 ± 0.8 | –0.6 ± 0.7‡ | –0.6 ± 0.8 | –0.2 ± 0.5* |
| Femur | –1.1 ± 0.9 | –0.6 ± 0.7** | –0.8 ± 0.7 | –0.3 ± 0.5 |

Data presented as mean ± SD; Comparison of areal bone mineral density pre- and post-treatment in the same sex group : *=p<0.05 †=p<0.01 ‡=p<0.001

BMD= bone mineral density; NOF=neck of femur; BMI = body mass index; Z-score = standard deviation score calculated from age and sex matched healthy controls

bone loss occurs soon after delivery, but it may not translate into osteoporosis in the long run.[30] We also found a negative correlation between duration of lactation and bone density. Some studies have shown a reduction in BMD with lactation, if continued beyond 3 months.[30] One possible reason why we found this correlation is that the duration of lactation seen in our cases was unusually prolonged. A high calcium intake is not known to prevent this bone loss and the time period required for return of BMD to baseline is also debatable.[31-33]

Serum $T_4$ correlated negatively with Z-scores at LS,NOF, trochanter and Ward's area. Similar findings have been obtained by some investigators[34] while others didn't find any correlation.[35] In our study there was improvement in BMD and Z-scores at lumbar spine and all parts of femur, 6.2 % and 8.8%) in men and 10.6% and 7.3% in women respectively, 6 months after correction of hyperthyroidism. Improvement was more at femur than at LS. Incomplete recovery (3.7% to 6.6% after 1 year) appears to be the rule.[7,36] However, full recovery has been reported after 3 years of euthyroid status.[6] Modality of treatment of thyrotoxicosis appears to be less important than the functional status of the thyroid.[4,5] However, worsening of BMD (T-score and Z-score) at forearm has been reported after 1 year of medical therapy despite improvement at other sites.[37] At the follow up none of the patients had low Z-score at femur, but 21% women and 14% men still had low Z scores (< –2) at LS. It appears that recovery is better at NOF post-treatment.

Hypercalcaemia {serum corrected calcium >10.5mg/dl (2.62 nmol/L)} was present in 10 (25%) patients. We did not observe any correlation between serum calcium, $T_4$ or BMD at any site. Following treatment only 1 out of 21 (4.7%) of patients had Hypercalcaemia. The mean fall in serum calcium was 4.2 % was similar to that reported in a previous study.[38]

Hypercalciuria was noted in 42.5% of patients at the time of diagnosis. Calciuria did not correlate to either serum $T_4$ or BMD at any site. Hypercalciuria, correlating to thyroid functions and to cortical osteoclastic bone resorption, has been

reported to be present in upto 64% of cases.[39,40] It is not ameliorated by calcium restricted diet. Only 2 out of 21 (9.5%) of patients had hypercalciuria following treatment, similar to reductions reported in literature.[38]

Fractional excretion of calcium (FECa) fell post treatment. One possible cause could be an increase in serum PTH which increases tubular reabsorption of calcium and decreases that of phosphorus. A decrease in the 24-hour urinary excretion of calcium post-treatment has been reported in hyperthyroid patients[39] In addition to the fall in daily urinary phosphate excretion on restoration of the euthyroid state as observed in another study[41] the fractional excretion of phosphorous (FEPi) also fell despite a rise in serum PTH post treatment. Contrary to what has been observed by us, the fractional excretion of phosphate would normally have been expected to have gone up as the PTH rises. High renal blood flow and glomerular filtration rate in patients with hyperthyroidism along with the increased mobilization of mineral from bone may account in part for the hyperphosphaturia[41,42] but does not explain an initially higher fractional excretion of phosphate and its subsequent fall on restoration of the euthyroid state. A decrease in the fractional excretion of phosphorous post-treatment might be suggestive of a direct effect of thyroid hormone to promote renal tubular wastage of phosphorous.

Our patients had normal serum phosphorous both pre-and post-treatment. Other investigators have also reported a normal phosphorus level[43] while some others have found elevated serum phosphorus,[44,45] possibly due to suppressed parathyroid hormone and enhanced mobilization from bone and soft tissues.[10] We did not find any correlation between serum phosphorous and BMD.

We also found elevated serum alkaline phosphatase, which remained elevated post treatment. We did not find any correlation between alkaline phosphatase and serum 25(OH)D, hence raised levels of serum alkaline phosphatase could not be ascribed to vitamin D deficiency alone. Other groups have also reported high alkaline phosphatase activity, mainly bone specific fraction, in patients with thyrotoxicosis.[10,40,46] We found a negative correlation between serum alkaline phosphatase and BMD at Ward's area. A negative correlation of BMD at LS with bone specific alkaline phosphatase at baseline[44] has been reported. High alkaline phosphatase, at the end of one year, seems to predict low bone mass[28]

We found low serum 25(OH) D [18.9 ± 6.7 ng/mL (47 ±17nmol/L) and 17.1 ± 9.5 ng/mL (43 ± 24 nmol/L)in women and men respectively], similar to our previous reports on non-thyrotoxic population from the same geographical region. Most of the studies on bone density in thyrotoxic patients, conducted in industrialized nations, have sparse and conflicting data on serum 25(OH)D, ranging from low[48,49] to normal serum 25(OH)D.[35] However, absence of any correlation between serum 25(OH)D and bone histomorphometry precludes any contribution of vitamin D deficiency to thyrotoxic bone disease.[10] We did not find any correlation between serum 25(OH)D and BMD.

Contrary to the expectations, we found normal serum PTH among our subjects despite having vitamin D deficiency or insufficiency. Normal serum PTH can possibly be attributed to concomitant Hypercalcaemia. Post-treatment rise of serum PTH (due fall in serum calcium) despite having no change in serum 25(OH) D supports this conclusion. Similar rise in serum PTH, post treatment, has been reported in thyrotoxic subjects having normal serum 25(OH)D, both at baseline and post treatment.[38] Thyrotoxicity is thus associated with changes inmarkers of bone disease and mineral metabolism along with loss of BMD. Most of these changes are reversible with return to euthyroid status. High Wayne's thyroid score is a good pointer towards severe thyrotoxic bone disease.

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